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XpressDNA Mycobacterium (MTB) Kit

Protocol for isolating high quality genomic DNA from mycobacterium cells using XpressDNA Mycobacterium kit.

Process Flow

Kit Contents

Components	Storage Conditions	Shipping Conditions
MTB Enzyme Buffer	RT	RT
MTB Lysis Buffer	RT	RT
Proteinase K	2 - 8 °C	RT
Proteinase K Buffer	2 - 8 °C	RT
RNase A	2 - 8 °C	RT
MTB MagNa Mix	RT	RT
MTB Wash Buffer 1	RT	RT
MTB Wash Buffer 2	RT	RT
MTB Elution Buffer	RT	RT
MagNa Stand (optional)	RT	RT

* RT denotes 15 - 25°C.

Materials not provided with the kit

1. 100% Ethanol to Wash Buffers as indicated on the bottle.
2. Water bath/heat block at 56°C.
3. Reconstitute Proteinase K with Proteinase K Buffer and store at 2 – 8°C.
4. 1X PBS (pH 7.4)
5. Lysozyme stock (20 mg/ml, to be prepared freshly).

Important

Pay attention to standard lab practices and safety information before beginning the procedure. For more information, refer the appropriate Material Safety Data Sheet (MSDS) available from the product supplier or download from our website http://www.maggenome.com/

Technical Support

For any product related queries please write to us on info@maggenome.com, sales@maggenome.com, support@maggenome.com.

Protocol

Bacterial Lysate Preparation	1. Take **1ml of overnight grown Bacteria culture (OD600 > 1)** and centrifuge at 10,000 rpm for 5 minutes at room temperature. Discard the supernatant. 2. Add **500 µl of 1X PBS** and resuspend the pellet completely by pipette mixing. 3. Centrifuge at 10,000 rpm for 5 minutes at RT and discard the supernatant. 4. To the pellet, add **190 µl of MTB Enzyme Buffer** and completely resuspend the pellet by pipette mixing ***(ensure proper resuspension of pellet).*** 5. Add **10 µl of freshly prepared lysozyme** and mix thoroughly by pipette ***(Pipette mixing 3 - 4 times).*** 6. Incubate at 37°C for 60 minutes. 7. Add **800 µl of MTB Lysis Buffer** and mix thoroughly by pipette ***(Pipette mixing 10 - 15 times).*** 8. Add **20 µl of Proteinase K** and quick vortex. 9. Incubate at 56°C for 90 minutes or overnight lysis ***(Lysis time can vary depending on strain) (Pipette mix completely every 30 minutes).*** ***Note: For better dispersion, use 200 µl pipette for re-suspending the pellet*** 10. Add **10 µl of RNase A** and quick vortex. 11. Incubate at room temperature for 15 minutes. 12. Centrifuge at 14,000 rpm for 5 minutes at RT. 13. Transfer the supernatant to a fresh 1.5 ml tube.

DNA Binding	***(Note: Vortex the MTB MagNa Mix thoroughly before the next step)*** 14. Add **400 µl of MTB MagNa Mix** and gently invert the tube 10 - 12 times to mix properly. Do not vortex. 15. Incubate the samples at RT for 5 minutes. 16. Place the tube on a MagNa Stand until the solution becomes clear. 17. Carefully discard the supernatant without removing the tube from the MagNa Stand. Ensure the magnetic nanoparticles are not disturbed.

DNA Washing	18. To the magnetic nanoparticles, add **500 µl of MTB Wash Buffer 1**, remove the tube from the MagNa Stand and resuspend by thorough pipette mixing to ensure complete dispersion of the particles. ***(Note: Use 200 µl pipette for better resuspension of the pellet.)*** 19. Place the tube back on the MagNa Stand for 30 - 60 seconds till the solution becomes clear. 20. Discard the supernatant without removing the tube from the MagNa stand. Ensure the magnetic nanoparticles are not disturbed. 21. Add **500 µl of MTB Wash Buffer 2** & gently invert mix the tube 5 – 6 times without

	removing from the MagNa Stand ***(surface wash only).*** 22. Discard the supernatant without removing the tube from the MagNa Stand. 23. Repeat steps **21 - 22.** 24. Air dry the magnetic nanoparticles without removing the tube from MagNa Stand for 10 - 15 minutes without over drying them.

DNA Elution	25. After drying, remove the tube from the MagNa Stand. 26. Add **50 - 100 µl of MTB Elution buffer** and resuspend the magnetic nanoparticles by pipette mixing thoroughly. 27. Incubate at 56°C for 5 minutes with intermittent tapping**.** 28. Place the tube back on the MagNa Stand for 5 minutes or until the solution becomes clear. 29. Carefully transfer the supernatant containing DNA to a sterile 1.5 ml tube, without removing the tube from the MagNa Stand. Ensure the magnetic nanoparticles are not disturbed. 30. Discard the magnetic nanoparticles in the appropriate hazard container.

Note: In the elution step, if the Magnetic particles take more than 10 minutes for clearing, spin the tubes at 14,000 rpm for 5 minutes, place on MagNa Stand until solution clears and then collect the supernatant with pure DNA.

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Troubleshooting Guide

Observation	Possible causes	Suggested Solution
Low DNA Yield or Poor Quality	Low Culture Density	Use bacterial culture with OD_{600} above 1.
		For better yield, use overnight grown cell culture or cell pellets stored in proper conditions.
	Poor Cell Lysis	Cells may not be dispersed properly in lysis buffer. Disperse the cells by adequate pipette mixing.
		For bacterial strains with high DNA content, the lysate may appear viscous. For such samples thorough pipette mixing is recommended.
		Add required volume of Proteinase K into the Lysis buffer. Ensure optimal lysis temperature. Proteinase K solution should be stored at 2 - 8°C.
	Ethanol is not added to wash buffers	Add 100% ethanol to wash buffers as suggested on the bottles.
RNA contamination	RNase A not added	Add RNase A as per the protocol.
Poor performance of extracted DNA in downstream applications	Ethanol carryover	Air dry the Magnetic particles after the washing steps to remove ethanol completely, but do not over dry the pellet.
	Salt carryover	Ensure that the correct amount of ethanol is added to the Wash Buffers and the two wash steps are performed with Wash Buffer 2.